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Acute Promyelocytic Leukemia (APL) With An Unusual Cytogenetic Presentation

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DISCUSSION

Erol Yorulmazoglu, MD (Hematology/Oncology Fellow)

We present a patient with APL because of the therapeutic implications of the singular chromosomal rearrangement present in his leukemic cells. Our patient is a 60-year-old male who presented with easy bruisability, gum bleeding, weakness, decreased appetite, and night sweats. He is a retired admiral without significant medical history except that he was exposed to Agent Orange while he was in Vietnam. His physical was unremarkable except for multiple ecchymosis of the skin.

Admission CBC consisted of WBC 1,800/μL, Hgb 8.5 g/dL, Hct 25.1%, Plt 10,000/μL with the differential count showing neutrophils 5%, bands 4%, myelocytes 4%, promyelocytes 67%, blasts 7%, and lymphocytes 13%.

Sefer Gezer, MD (Hematologist)

Initial work-up also included bone marrow aspirate and biopsy demonstrating hypercellular marrow with 64% promyelocytes which was consistent with ANLL-M3 (APL). Immunophenotyping studies revealed 75% CD13, 0% CD14, 93% CD33, 0% CD34, and lymphoid markers were negative. His coagulation profile showed PT, 13.2 sec; INR, 1.3; APTT, 25 sec; fibrinogen, 435 mg/dL; FSP >40 μg/mL; D-dimers, 2.0–4.0 μg/mL; FV and FVIII:C were within normal limits.

Erol Yorulmazoglu, MD

Subsequently the patient was admitted with a diagnosis of ANLL-M3 (APL) with neutropenic fever. The treatment then included all-trans retinoic acid (ATRA) 45 mg/m^2, and broad antibiotic coverage with ceftazidime, tobramycin, vancomycin.

Stephanie A. Gregory, MD (Hematologist)

In 1949 French hematologists first described exaggerated bleeding complications in certain leukemic cases [1]. It was Hillstad [2] who, in 1957, first labeled this form of AML as ''promyelocytic leukemia.'' It presents with the commonly encountered chromosomal abnormality, the translocation t(15;17). Essentially APL is identified by the morphology of the leukemic cells, the 15;17 translocation, and coagulopathy. Other characteristics include younger median age of onset, leukopenia or pancytopenia, high sensitivity to anthracyclines, and a unique response to ATRA.

Erol Yorulmazoglu, MD

During the initial course of hospitalization the patient remained febrile. However, as he developed mild edema and leukocytosis, dexamethasone was added, since we thought that this condition might have been the ''ATRA syndrome.'' But ATRA was continued. Eventually he defervesced and the edema resolved. Dr. Gezer, would you comment on the adverse effects of ATRA?

Sefer Gezer, MD

There are two main complications of ATRA that I will mention. The first is hyperleukocytosis, which in itself can lead to pulmonary and CNS toxicity. Hyperleukocytosis from ATRA use can be managed by initiating cytotoxic chemotherapy earlier.

The second complication is the retinoic acid syndrome, which can be seen in up to 25% of APL patients on ATRA. The onset is within 2 to 28 days of treatment with ATRA. Symptoms and signs can include fever, respiratory distress, pulmonary infiltrates, pleuropericardial effusion, edema, and hypotension. For severe symptoms, ATRA should be discontinued and high dose steroids should be added. However, low dose steroids can

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TABLE I. Myeloid Response to ATRA

Date	Day	WBC (μL)	Blast	Promyelo	Myelo (%)	Meta	Band	Segs
9/20	1	1,800	6	67	4	0	4	5
9/21	2	6,600	5	77	3	1	0	3
9/22	3	3,100	0	14	27	12	8	10
9/23	4	6,800	0	4	32	23	9	7
9/24	5	10,200	1	8	35	24	4	2
9/25	6	13,900	2	4	54	16	0	5

be started without discontinuation of ATRA at the earliest signs of retinoic acid syndrome.

Erol Yorulmazoglu, MD

When the patient had a rapid rise in his total WBC count, chemotherapy with idarubicin was initiated. He also developed transient pericarditis, but this resolved spontaneously. He had no evidence of bleeding throughout hospitalization but following chemotherapy, the patient again required hospitalization for neutropenic fever. Consolidation chemotherapy with (3 + 7) idarubicin and cytarabine (Ara-C) was given in 3 consecutive courses. The patient remains in complete remission.

Stephanie A. Gregory, MD

In the pre-ATRA period, cytotoxic chemotherapy was used for induction as well as consolidation. Complete remission (CR) durations of up to 24 months have been observed in APL treated with single agents, such as idarubicin [3] and daunorubicin [4,5]. CR rates of 68 to 80% have been observed [5]. With the addition of ATRA, CR rates over 90% have been seen [6].

Dr. Yorulmazoglu, would you elaborate on the use of ATRA in APL patients?

Erol Yorulmazoglu, MD

ATRA therapy was initiated by Chinese and French investigators. Many of their studies [7,8] demonstrated that ATRA had high efficacy in inducing differentiation of APL cells. A molecular study by Lo Coco et al. [9] showed maturation of myeloid cells 2 to 3 weeks after starting ATRA and total disappearance of APL cells after 5 to 8 weeks by using Southern blot analysis.

However, the success of ATRA is dose dependent: the peak concentration time after an oral dose of 45 mg/m^2 is 60 to 210 min, with maximum concentrations in the range of 0.003 to 2.5 mg/mL [10]. At concentrations greater than 0.1 mM, ATRA has been shown to decrease the expression of PML-RARα oncoprotein in APL cells within 12 hours [11]. ATRA is given at 45 mg/m^2 until CR is achieved; usually 40 to 45 days. A Chinese clinical trial has shown that 15 mg/m^2 ATRA, which reached 0.1 mM concentration, was as efficacious as the usual 45 mg/m^2 dosing [12].

In the study by Fenaux et al. [6] ATRA provided CR rates over 90% in untreated patients. In the first patients who relapsed after chemotherapy alone, CR rates were also comparable (85–90%) when ATRA was used for second induction. In order to maintain second CR, however, bone marrow transplant is required. Outcomes of second CR following ATRA use with subsequent intense chemotherapy and bone marrow transplant remain to be seen. Patients who have relapsed after ATRA therapy or shortly after its discontinuation may be resistant to its reapplication. In contrast, patients who relapsed 4 to 25 months after discontinuation of ATRA will still be sensitive to the agent. Unfortunately, results with ATRA for second and subsequent relapses are disappointing.

It is believed that ATRA modulates the negative function of PML-RARα oncoprotein, which results from the fusion of the PML gene and the retinoic acid receptor alpha gene. APL cells that do not demonstrate the PML-RARα fusion gene are said to be ATRA resistant [13].

Remissions with ATRA alone, however, are brief [6]. Addition of anthracycline based induction chemotherapy is necessary to have longer CR duration [7,14].

Stephanie A. Gregory, MD

Poor prognostic factors include older age, leukocytosis (also an indicator for a shorter remission), severe coagulopathy, and the microgranular variant of APL. Let us hear from Dr. Gezer about the bleeding complications that APL patients can exhibit on presentation.

Sefer Gezer, MD

Life-threatening bleeding diatheses is included to DIC or primary fibrinolysis, associated with t(15;17). The mechanisms responsible for these events are still debated. However, DIC and primary fibrinolysis are believed to be the result of the release of procoagulant activities, plasminogen activators, and lysosomal neutrophil enzymes from APL cells. In the presence of ATRA, primary fibrinolysis may subside within 1 week but DIC may persist for up to 2 to 3 weeks. Moreover, hypercoagulability that may be present as a result of these complications can in itself predispose the patient to thromboembolic phenomena. Life-threatening bleeding complications are very rare with ATRA treatment as compared to conventional chemotherapy.

Erol Yorulmazoglu, MD

We know that in 1976, Rowley and Golomb [15] first described an abnormality in chromosome 17 of APL patients. Now we know that these patients present with a reciprocal translocation between chromosomes 15 and 17.

Wei-Tong Hsu, MD (Geneticist)

Acute promyelocytic leukemia is characteristically associated with a specific cytogenetic abnormality, t(15;17) [15], and it has been suggested that practically all APL patients would be found to have this specific abnormality if optimal techniques are employed [16]. Molecular analysis has revealed that this translocation results in the juxtaposition of the retinoic acid receptor alpha gene (RARα) in the chromosome region of 17q11.2-21 and the PML gene in the chromosome region of 15q22, giving rise to a new PML-RARα hybrid gene on chromosome 15. Recent evidence indicates that the RARα gene is part of the nuclear steroid/thyroid hormone receptor superfamily, and that the PML gene encodes a protein that contains a cysteine-rich region typically present in DNA-binding proteins. It is likely to be a transcription factor of a zinc finger [17]. The PML-RARα hybrid protein apparently acts in a dominant manner to interfere with promyelocyte differentiation [18], perhaps through a competitive inhibition of RARα or PML; the exact mechanism is still unknown.

This patient with APL had the PML-RARα gene fusion located on chromosome 17 as a result of an unusual chromosome rearrangement between chromosomes 15 and 17. The findings were documented by conventional cytogenetics and fluorescent in situ hybridization (FISH).

Sefer Gezer, MD

Dr. Hsu, would you discuss how you studied the cytogenetics of this patient?

Wei-Tong Hsu, MD

Initial cytogenetic analyses of 20 metaphases from 24-hour and 48-hour unstimulated bone marrow cultures revealed 3 cell lines. The first cell line (8/20 metaphases) contained a derivative chromosome 17 that could have been derived from the insertion of part of chromosome 15 between q15 and q22 into the long arm of chromosome 17 at the region of q21. The second cell line (7/20 metaphases) contained 47 chromosomes with trisomy 8 in addition to the derivative chromosome 17. The remaining cell line (5/20 metaphases) had a 46,XY normal male karyotype.

After initial treatment with ATRA, follow-up cytogenetic studies revealed a 46,XY normal male karyotype.

Subsequent studies with FISH used the LSI™ PML-RARα translocation probe (Vysis, Inc.) and chromosome 15 and 17 painting probes (Oncor, Inc.). They were performed to define both the composition of the derivative chromosome 17 and to determine whether the PML and RARα sequences known to be juxtaposed as a critical genetic event in APL were involved in this rare chromosome rearrangement. FISH analysis using dual color LSI™ PML-RARα probe and CEP 15 probe (chromosome 15 satellite probe) (Vysis, Inc.) simultaneously demonstrated that the PML-RARα fusion signal was unexpectedly present on chromosome 17 instead of chromosome 15. Further FISH study using paints specific for chromosome 15 and 17 separately revealed that the derivative chromosome 17 was composed of chromosome 17 sequences at both its short arm and the end of its long arm. One sequence of chromosome 15 was also present interstitially.

Erol Yorulmazoglu, MD

It is important to recognize the clinical presentation and to make a timely diagnosis of APL, since it is considered a medical emergency. The diagnosis is made by the morphologic evaluation of the bone marrow, the clinical presentation, and the detection of the PML-RARα fusion gene, which is pathognomonic of APL. Diverio et al. [13] describe a rapid RT-PCR technique which allowed them to detect the PML-RARα fusion gene within 6 hours, and as a result, patients were able to start their appropriate treatment more rapidly.

ATRA has successfully promoted maturation of the promyelocytes and in the process the malignant cells are transformed into benign ones. Potential problems, such as RA syndrome, may occur during the course of ATRA therapy. Also, during this treatment, the WBC count can rise rapidly, as it did in this case, prompting the addition of induction chemotherapy.

Wei-Tong Hsu, MD

Our observations in this case suggest that the juxtaposition of a PML/RARA fusion gene was located on chromosome 17 as the result of a chromosome 15 insertion into chromosome 17. A case with a similar but more complex chromosome insertion has been reported [19] which demonstrated that the *der* (17) consisted of a complex rearrangement with duplication of both RARα and PML, insertion of chromosome 1 sequences, and double insertion of chromosome 15 sequences. However, we can not exclude the possibility of PML-RARα gene fusion arising from 2 successive translocations, which includes a classical t(15;17) and a subsequent translocation between the regions of chromosomes 15q15 and 17q21 to give rise to the unusual chromosome rearrangement with the aberrant location of a PML-RARα fusion gene. It has been reported that in APL patients with complex chromosome translocations, cytogenetic and FISH analysis revealed secondary chromosome changes occurring after standard t(15;17) [20].

In APL patients, RARα is fused to PML in the great majority of patients as a result of the chromosome translocation 15;17. It has been documented in the literature that in APL patients with cytogenetically defined variant translocation between 15q, 17q, and a third chromosome, or between 17q and a chromosome other than 15, a PML-RARα fusion gene is also involved [19,21,22,

23,24,25,26], although PML-RARα can take place on chromosome sites other than chromosome 15q22. However, a small subset of APL patients (1–2%) had a different gene fusion, PLZF (promyelocytic leukemia zinc finger)-RARα, resulting from the variant translocation (11;17) [23,24,27,28]. A third translocation, t(5;17), in which the NPM (the nucleolar phosphoprotein nucleophosmin) gene is fused to RARα, has been described. Clinically, patients with t(11;17) and t(5;17) are different from typical or variant t(15;17) APL patients. They respond poorly to ATRA treatment [29].

Trisomy 8 has been reported as the most frequent (17%) secondary abnormality in APL at diagnosis. The possible prognostic value of additional chromosomal changes remains to be evaluated [30].

We conclude that FISH provides an efficient alternative tool to study a PML and RARα fusion. For clinical purposes, it is necessary in APL patients to determine the presence or absence of a PML-RARα gene fusion using the FISH technique to identify all variant (15;17) translocations.

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